Title: Improvements in or Relating to Inhibition of Viruses

Field of the Invention

The present invention relates to a method of inducing mutations in viruses, a method of inhibiting the replication of viruses, pharmaceutical compositions for use in inhibiting the replication of viruses, and the use of various compounds in the preparation of medicaments to inhibit viral replication. The invention specifically applies to RNA viruses, that is, viruses which have an RNA genome or which replicate via an essential RNA intermediate.

Background of the Invention

RNA viruses are responsible for many diseases of man and animals. Examples of RNA viruses which are human pathogens include influenza virus, poliovirus, rhinovirus and HIV. A specific example of a pathogenic DNA virus which replicates via an essential RNA intermediate is hepatitis B virus (HBV).

Very few effective antiviral agents are currently available. Certain compounds which are moderately effective against HIV are deoxynucleoside analogues. These act by inhibiting HIV replication by acting as "chain terminators" i.e. causing termination of HIV reverse transcriptase-mediated DNA synthesis. However the efficacy of such drugs is limited because of the emergence of resistant strains of viruses. RNA viruses in general, and HIV in particular, have a very high mutation rate during replication, and this high mutation frequency enhances the likelihood of resistant strains emerging.

Recently the idea has developed that RNA viruses may be close to the "edge of viability". That is, the mutation frequency of such viruses is so high that a comparatively modest increase in mutation frequency may be sufficient to render the great majority of the viral population non-viable, due to the presence of deleterious mutations at essential loci in the viral genome. This well-known concept is known as "error catastrophe" and results with the wide spectrum antiviral ribavirin in the context of poliovirus strongly suggest that the

concept is well-founded (Crotty *et al*, 2000 Nature Medicine 6, 1375-1379; Crotty *et al*, 2001 Proc. Natl. Acad. Sci. USA 98, 6895-6900; Sierra *et al*, J. Virol., 2000, 74, 8316-8323).

Loeb *et al*, (WO 98/18324 and US 6,063,628) disclose the use of ribonucleoside analogues to increase the mutation rate in (and thereby inhibit the replication of) RNA viruses such as HIV or HCV. Loeb *et al* state that the ribonucleoside analogue may typically be an analogue of cytidine, uridine, adenosine or guanosine, but that analogues of cytidine or uridine (i.e. pyrimidine analogues) are preferred (US 6,063,628; column 3 lines 44-45). Loeb *et al* do not specifically refer to many purine nucleoside analogues, but adenosine analogues specifically mentioned include: 1,N^6-ethenoadenosine, 3-methyladenosine and N^6-methyladenosine. Guanosine analogues specifically mentioned include 8-hydroxyguanosine, O^6-methylguanosine, O^6-ethylguanosine, O^6-isopropylguanosine, 3,N^2-ethenoguanosine, O^6-alkylguanosine, 8-oxo-guanosine, 2,N^3-ethenoguanosine, and 8-aminoguanosine.

Interestingly, neither WO 98/18324 nor US 6 063 628 contain any data from experiments performed by the inventors to support the claims made therein. Only one experiment is described in which HIV is passaged *in vitro* in the presence of either 5-hydroxyuridine or 5-bromocytidine. The results after 4 passages are shown in Figure 3 of US 6 063 628: no decline in viral titer is apparent in the Figures.

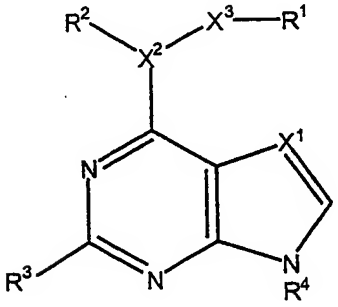
The content of all documents mentioned in this specification is incorporated herein by reference.

Summary of the Invention

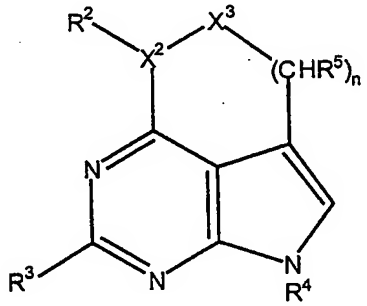
The present invention relates to certain nucleoside analogues which the present inventors, in contrast to the data presented by Loeb *et al*, have found to be effective in inhibiting RNA virus replication, even within 4 passages *in vitro*.

In a first aspect the invention provides a method of inhibiting the replication and/or increasing the mutation rate of an RNA virus, the method comprising administering an RNA nucleoside analogue to a cell infected by an RNA virus (as herein defined), the analogue being incorporated by a polymerase into an RNA copy of the viral genomic nucleic acid molecule, wherein the nucleoside analogue conforms to the general formula I or II below:

I



II



where:

n = 1-4, preferably 2-4,

X^1 = N or CH or CR^5

X^2 = N or S or CR^5

X^3 = NR^6 or O or S or R^6 when X^2 = N, or X^3 = NR^6 or R^6 when X^2 = S, and X^3 is absent when X^2 = CR^5

R^1 = H or alkyl or aryl or alkaryl or acyl

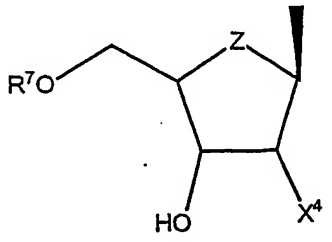
R^2 = H or alkyl or aryl or alkaryl or acyl; when X^2 = S, R^2 is absent;

R^3 = H or NR^5R^6 or $NR^5NR^5R^6$ or NR^5OR^5

R^5 = H or alkyl or alkenyl or alkynyl or aryl or alkaryl or acyl

R^6 = H or alkyl or alkenyl or alkynyl or aryl or alkaryl or acyl and

R^4 = H or



wherein

$Z = O$ or S or CH_2 or CHF or CF_2 or NR^5

$X^4 = OH$ or F

$R^7 = H$ or PO_3^{2-} or $P_2O_6^{3-}$ or $P_3O_9^{4-}$ or a masked phosphate derivative.

Alkyl groups, if present, are preferably methyl groups (desirably unsubstituted). Aryl groups, if present, are preferably phenyl groups, substituted or unsubstituted. Desirably no more than one aryl or alkaryl group is present in a molecule according to the general formulae. Conveniently at least one of R^1-R^6 is H and preferably at least two of R^1-R^6 are H.

A masked phosphate derivative is a modified phosphate group in which the negative charge(s) which would normally be present in an unmodified phosphate group are reduced or (more preferably) entirely neutralized by additional moieties. This has the benefit of facilitating transport of compounds comprising the modified phosphate group across a lipid membrane (e.g. across a cell membrane). Examples of masked phosphate derivatives are bis-POM/bis-POM PMEA (see Delaney *et al*, 2001 Antiviral Chemistry and Chemotherapy 12, 1-35), *cyclo*Sal (Meier *et al*, Eur. J. Org. Chem. 1998, 837) and SATE (Lefebvre *et al*, J. Med. Chem., 1995, 38, 3941-3950). (SATE is an abbreviation of S-acyl thioethyl).

For present purposes an "RNA virus" is considered to include all viruses with an RNA genome (encompassing both "conventional" RNA viruses and retroviruses) and any virus which requires a genomic RNA intermediate for the purposes of replication. Examples of relevant viruses include ortho- and paramyxoviruses, poliovirus, rhinovirus, retroviruses (especially HIV-1 and HIV-2), hepatitis B and C viruses (HBV and HCV respectively), rotaviruses, flaviviruses (e.g. West Nile virus) and certain arboviruses (e.g. Dengue Fever virus).

"Conventional" RNA viruses include both negative and positive stranded ss (single stranded) RNA viruses and ds (double stranded RNA viruses). In particular, a

"conventional" RNA virus may be defined as a virus having a single or double stranded RNA genome which encodes a viral RNA polymerase. An extensive, but not necessarily exhaustive, list of RNA viruses is shown in Table 1 in the Appendix. Those whose hosts are vertebrates, (especially mammalian vertebrates, and in particular man or domesticated mammals) are particularly suitable for inhibition by a pharmaceutical composition in accordance with the invention.

The invention encompasses the administration of a ribonucleoside analogue (that is, a base analogue covalently joined to a ribosyl residue) to an infected cell. The administered ribonucleoside analogues may be converted to the corresponding ribonucleotide analogues intracellularly by known enzymes. However it is also possible to perform the invention by administering the base analogue (without an attached ribosyl residue), which base analogue is then converted by phosphoribosylation (*in vivo* if administered to a living multicellular organism, or intracellularly if administered to a cell *in vitro*) into a ribonucleotide analogue. Equally the invention encompasses within its scope the administration of a ribonucleotide analogue (that is, a ribonucleoside analogue esterified to a phosphate group, or a di- or tri-phosphate). For the purposes of economy, the compounds of use in the invention are referred to as ribonucleoside analogues, although those skilled in the art will appreciate that the general formulae presented above encompass both base analogues and ribonucleotide analogues, and unless the context dictates otherwise, the term "ribonucleoside" analogue is intended to embrace both base analogue and ribonucleotide analogue. It is generally preferred that the base analogue incorporated in the ribonucleoside analogue is a purine base analogue, which term specifically includes 7-deaza purine analogues.

In some instances it may be preferred to perform the invention by use of base analogues, especially in preference to ribonucleoside analogues, since these may be better absorbed by mammalian subjects following administration *in vivo*.

Compounds for use in the invention and in accordance with the general formulae presented above are commercially available and/or are readily capable of being synthesised by those

skilled in the art using published protocols. Other compounds may be obtained by following the detailed teaching provided in the present specification.

In preferred embodiments Z is O. In the same or other preferred embodiments X^2 is N. In the same or other preferred embodiments X^3 is O or comprises N. In the same or other preferred embodiments X^4 is OH. Desirably, in one embodiment, Z is O, X^2 is N, X^3 is N or O and X^4 is OH. In an especially preferred embodiment Z is O, X^2 is N, X^3 is O, X^4 is OH and R_1 is alkyl, especially methyl.

Generally preferred are ribonucleotide analogues which have low cytotoxicity but high viral mutagenicity and/or high viral inhibitory activity. Particular examples of preferred ribonucleoside analogues include those illustrated in Figures 3, 7 and 11, and the corresponding base analogues and ribonucleotide analogues. Cytotoxicity can be readily assayed *in vitro*, by those skilled in the art using, for example, tissue cultures of relevant tissues (e.g. CHO cells, HeLa cells, CEM/O cells and the like). Typically an IC_{50} value can be determined (that is, the concentration of the agent under investigation which causes 50% inhibition of growth of the tissue culture cells relative to untreated control cultures). The amount of inhibition of growth may be estimated by any suitable means e.g. incorporation of tritiated thymidine; time taken for cultures to reach confluency, etc.

Anti-viral activity may be measured *in vivo* or *in vitro*. Suitable assay methods will be apparent to those skilled in the art with the benefit of the present disclosure. In particular, for example, viral inhibition may be measured *in vitro* by using plaque reduction assays, and determining for example the IC_{50} concentration of the compound under test (i.e. the concentration which reduces by 50% the number of viral plaques formed in a monolayer of susceptible cells after a fixed length incubation, relative to virus grown in the absence of the test compound).

The ribonucleoside analogue having the structure shown in Figure 11, which compound has the full name 2-amino-6-methoxyamino-9-β-D-ribofuranosylpurine, abbreviated for simplicity as rK, and the corresponding base analogue K and ribonucleotide analogue rKP

(which expression incorporates in particular mono-, di- and triphosphates) may be particularly useful. The di- and triphosphates may be referred to as rKDP and rKTP. The inventors have found that rK is active in reducing viral titer, especially the titer of HIV-1 when the virus is grown *in vitro* in tissue culture.

In order to be effective, the ribonucleoside analogues of the invention need to be incorporated into the RNA copy of the viral genomic nucleic acid with reasonable efficiency and must therefore be recognisable as a suitable substrate by the relevant RNA polymerase inside the host cell. For "conventional" RNA viruses this is an RNA polymerase encoded by the virus. For retroviruses, the relevant RNA polymerase is the RNA polymerase encoded by the host cell. Generally speaking, viral RNA polymerases are less faithful and less discriminating than host cell RNA polymerases and will be more likely to utilise the ribonucleoside analogues as substrates after *in vivo* conversion (when required) to triphosphates. Accordingly the pharmaceutical composition, method, and other associated aspects of the invention are preferably intended and adapted for use in the prevention and/or treatment of infections by conventional RNA viruses which encode a viral RNA polymerase.

The inventors have additionally made the surprising discovery that certain ribonucleoside analogues, preferably but not necessarily in accordance with general formulae I or II above, can inhibit retroviral transcription, which finding has not previously been suggested or in any way disclosed in the prior art. Without wishing to be bound by any particular theory, the inventors believe that this may be due to an inhibitory effect of the ribonucleoside analogue on transcription promoted by a 5' long terminal repeat ("LTR"), although the mechanism by which this inhibition might be mediated is unknown. Accordingly, preferred ribonucleoside analogues in accordance with the invention are those which exhibit the property of inhibiting viral production, e.g. by inhibition of transcription or by an error catastrophe mechanism. Methods of assaying compounds for such a property are disclosed herein and may be employed by those skilled in the art to identify ribonucleoside analogues possessing this desirable characteristic. The effect of inhibiting retroviral transcription is that there are fewer RNA copies of the viral genome

present in an infected cell: accordingly, at a given concentration of ribonucleoside analogue there are fewer RNA copies of the viral genome which are likely to escape incorporation of the mutagenic ribonucleoside analogue. A preferred compound in this regard is that denoted by the structure shown in Figure 2 referred to as rP, for simplicity (Moriyama *et al*, Nucleic Acids Research, 1998, 26, 2105-2111), and the corresponding base analogue (P) and the corresponding ribonucleotide analogue rPP (especially the triphosphate, rPTP).

It will be appreciated that increasing the mutation rate in the manner of the first aspect of the invention can, in accordance with the concept of error catastrophe, cause a significant increase in the number of non-viable viral particles produced, especially when the ribonucleoside analogue is present at an effective concentration for a plurality of cycles of viral replication, since mutations will accumulate in the viral genome over time. In contrast, although the ribonucleoside analogue will probably be incorporated into messenger RNA in the host cell (resulting in production of mutant polypeptides), mRNA is rapidly turned over and degraded and therefore will not accumulate mutations over time. Equally, the ribonucleoside analogue will generally not be incorporated into the DNA genome of the host cell or, if incorporated, will be removed by the "house-keeping" enzymes which are responsible for maintaining the integrity of the host cell genome. Accordingly, the method of the invention finds therapeutic application in the treatment of RNA virus infections.

Thus, in a second aspect the invention provides a method of treating an RNA virus infection in a human or animal subject, the method comprising administering to a subject infected with an RNA virus, an effective amount of a ribonucleoside analogue in accordance with general formula I or II.

In a third aspect the invention provides a pharmaceutical composition comprising an effective amount of a ribonucleoside analogue in accordance with general formula I or II in admixture with a physiologically acceptable excipient, diluent or carrier.

In a fourth aspect the invention provides a method of making a pharmaceutical composition, the method comprising mixing a ribonucleoside analogue in accordance with general formula I or II with a physiologically acceptable excipient, diluent or carrier. The method optionally includes the further step of packaging the composition in unitary dose form.

In a fifth aspect the invention provides for use of a ribonucleoside analogue according to general formula I or II in the preparation of a medicament to treat an RNA viral infection in a human or animal subject.

The ribonucleoside analogues of use in one or more of the various aspects of the invention will preferably be substantially soluble in water and be readily capable of entering virally-infected cells. Where the compound consists of a base analogue, the compound may generally be ribosylated and phosphorylated *in vivo*, or at least intracellularly. Where the compound is a ribonucleoside analogue it may typically be phosphorylated to form a ribonucleotide analogue. Possibly it is the ribonucleotide analogue which is integrated into the RNA genome of the RNA virus (or DNA virus which replicates via an essential genomic RNA intermediate), although it is important to note that the inventors make no assumption as to mode of action. Thus the active compound may be the base analogue and/or the ribonucleoside analogue and/or the ribonucleotide analogue. Specifically in respect of integrating retroviruses, such as HIV, the presence of the active compound incorporated by a cellular polymerase probably leads to mutation by the viral reverse transcriptase during DNA synthesis prior to integration into the host genome, which mutations are not recognisable by repair enzymes; over several cycles such mutations will accumulate.

Pharmaceutical compositions in accordance with the invention may be administered by any conventional route known to those skilled in the art. The preferred route is oral administration, but the composition may alternatively be administered, for example, intravenously, subcutaneously, transdermally, or via a rectal or intranasal route.

The composition may be administered as a solid (e.g. in the form of a tablet, pill, capsule, powder or the like) or may be a liquid (e.g. solution, suspension), semi-solid (e.g. a gel), aerosol or spray.

Physiologically acceptable excipients, diluents and carriers are well known to those skilled in the art of medical formulations and include, for example: saline, Ringer's solution, distilled water, dextrose solution, calcium carbonate, silicates, starches and modified starches and plant-derived polysaccharide gums and gels (e.g. xanthan gum; carrageenans and the like).

An "effective amount" of a ribonucleoside analogue or pharmaceutical composition comprising the same is understood to mean, for present purposes, an amount sufficient to cause a measurable decrease in the viral titer in suitable samples (e.g. blood, saliva, or tissue biopsy specimens) taken from the subject, or a measurable decrease in the amount of viral antigen detected in such samples; or a discernible amelioration in the symptoms of the viral infection experienced by the subject. Methods of obtaining suitable samples from a subject, and of analysing same to measure viral titer or viral antigen (e.g. by ELISA or other immunoassay) are well known to those skilled in the art.

The appropriate dose of the ribonucleoside analogue will depend on several factors, such as the body mass of the subject, level of toxicity (if any) of the analogue, the age of the subject and the severity of the viral infection (and/or any additional condition afflicting the subject). Guidance is given in US 6,063,628. Conveniently the dose of ribonucleoside analogue will be in the range 1mg/Kg body weight to 500 mg/Kg per day, preferably in the range 5mg/Kg - 250mg/Kg, more preferably 10mg-100mg/Kg.

Typically a dose at the lower end of the acceptable range is administered to the subject and, if there is no discernible improvement in the subject's condition, the dose may be increased if there are no contra-indications, until an effective dose is achieved. By such trial and error clinicians will readily be able to find an appropriate dose for any particular subject.

Advantageously the pharmaceutical composition in accordance with the invention may comprise more than one anti-viral agent. For instance, the composition may comprise a plurality of different ribonucleoside analogues, each being in accordance with general formula I or II defined above.

Additionally, or alternatively, the composition may comprise one or more antiviral agents which do not conform to general formula I or II. Examples include conventional antiviral agents such as ribavirin, AZT, HIV protease inhibitors, and compounds of the sort explicitly disclosed in US 6063628. The other aspects of the invention may conveniently reflect such embodiments.

Alternatively, the method of treating the subject may comprise separate administration of a further pharmaceutical composition comprising an additional anti-viral agent, such as those aforementioned, or a substance that reduces the intra-cellular concentration of the naturally-occurring ribonucleotide(s) with which the ribonucleoside analogue must compete for incorporation into the viral RNA genome.

In a further aspect the invention provides a composition suitable for application to a plant, for the purpose of preventing or treating an RNA virus infection of the plant, the composition comprising an RNA nucleoside analogue conforming to general formula I or II as defined elsewhere, the term "nucleoside analogue" also incorporating reference to a nucleotide analogue and a base analogue.

The composition will typically be applied to a plant by means of spraying a solution or suspension of the active antiviral agent (typically an aqueous solution or suspension). Conveniently the composition is supplied to a user in concentrated form and is diluted with water prior to application. Conveniently the composition will further comprise other substances conventional in the field of plant protection, to assist adherence of the composition to the plant to which the composition is applied and uptake of the active agent

by the plant. Such substances include, for example, surfactants and penetration enhancers, which are well known to those skilled in the pertinent art.

The list of viruses in the Appendix shows many RNA viruses which infect plants. In principle, any of these could be inhibited by the composition defined above. The invention thus also further provides a method of preventing and/or treating an RNA virus infection in a susceptible plant, the method comprising the step of applying to the plant an effective amount of a composition comprising an RNA nucleoside analogue conforming to general formula I or II defined above, and a method of making a plant protection composition for preventing and/or treating an RNA virus infection in a plant.

The invention will now be further described by way of illustrative example and with reference to the accompanying drawings, in which:

Figure 1 shows the structural formula of a deoxyribonucleoside analogue, dP;

Figure 2 shows the structural formula of a ribonucleoside analogue rP, the 'ribo' equivalent of the compound shown in Figure 1;

Figures 3-20 show the structural formula of various ribonucleoside analogues in accordance with general formula I or II identified above;

Figures 21 and 22 are graphs of p24 antigen (ng/ml) against time (in days);

Figure 23 is a schematic representation of a transcription system of use in screening ribonucleoside analogues for use in the present invention;

Figure 24 is a bar chart showing the amount of RNA transcript produced (in femtomoles) by a transcription system of the sort illustrated in Figure 23, in the presence or absence of a ribonucleotide analogue rPTP;

Figures 25A and 26A are images of PAGE analysis of nucleic acid extension assays performed using certain ribonucleotide analogues in accordance with the invention and Figures 25B and 26B are representations of the same results in bar chart form.

Figures 27 and 28 show the general structural formulae of certain masked phosphate derivatives of use in the invention.

EXAMPLES

Example 1 – Synthesis of Purine Ribonucleoside Analogues

The inventors synthesised several ribonucleoside analogues in accordance with general formula I or II, and also a ribonucleoside (N^4-hydroxycytidine) specifically mentioned by Loeb *et al* in US 6 063 628. For brevity the synthesised compounds are referred to herein as JA22-JA31. An additional compound, JA21 (Hill *et al*, Proc. Natl. Acad. Sci. USA, 1998, 95, 4258-4263), was synthesised and used as a control. JA21 is the deoxyribonucleoside equivalent of the ribonucleoside analogue JA22. JA29 is the compound indicated by Loeb *et al* as being useful in increasing the mutation frequency of RNA viruses (although no data are presented by Loeb *et al* in support of that assertion). The inventors also prepared a number of different base analogues (JA32-JA39). The table below (Table 1) indicates the systematic name of each of the compounds referred to as JA21-JA39, and also any trivial name if such a name has been used previously.

Table 1

Compound Number	Systematic Name	Trivial Name (if any)
JA21	6-(2-deoxy-β-D-ribofuranosyl)-3,4-dihydro-8H-pyrimido[4,5-c] [1,2] oxazin-7-one	dP
JA22	6-(β-D-ribofuranosyl)-3,4-dihydro-8H-pyrimido[4,5-c] [1,2] oxazin-7-one	rP
JA23	2-amino-N^6-methyladenosine	-
JA24	N^6-amino-9-β-D-ribofuranosyl-2, 6-diaminopurine	-
JA25	N^6-aminoadenosine	-
JA26	N^6-methoxyadenosine	-
JA27	N^6-amino-N^6-methyladenosine	-
JA28	N^6 hydroxyadenosine	-
JA29	N^4-hydroxycytidine	-
JA30	2-amino-N^6-hydroxyadenosine	-
JA31	2-amino-6-methoxyamino-9-β-D-ribofuranosylpurine	rK
JA32	N^6-hydroxyadenine	-
JA33	N^6-aminoadenine	-
JA34	N^6-amino-2,6-diaminopurine	-
JA35	N^6-amino-N^6-methyladenine	-
JA36	N^6-methyl-N^6-diaminopurine	-
JA37	N^6-methyl-N^6-methoxyadenine	-
JA38	N^6-methoxy-2,6-diaminopurine	K
JA39	N^6-methoxyadenine	Z

The structures of compounds JA21-JA39 are shown in Figures 1-19 respectively.

One of the main problems associated with nucleoside analogues as therapeutic agents is that they are often either only poorly phosphorylated by kinases to their monophosphate, or they are not substrates for the kinase at all. There are methods to generate nucleoside monophosphates in cells. One is to use the nucleobases, for example analogues JA32-JA39. These nucleobases may then be converted *in vivo* or *in vitro* to their nucleoside monophosphates by nucleoside phosphorylases (for a review see Pugmire and Ealick, Biochem. J., 2002, 361, 1-25). An alternative method requires the use of masked monophosphates, such as SATE-, cyclosal- or PMEA-derivatives. Nucleoside mono-, di-

or tri-phosphates may also be delivered into cells using transfection agents, such as liposomes (e.g. any commercially available liposome should, in principle, suffice – the inventors have used DMRIE-C, from Gibco BRL, as a specific example).

As examples of compounds of use in accordance with the present invention and in accordance with general formula I or II, JA23-JA31 (except JA29) were synthesised from 6-chloro-9-β-D-ribofuranosylpurine or 2-amino-6-chloro-9-β-D-ribofuranosylpurine (Aldrich). These were treated with the following available reagents: hydroxylamine hydrochloride, methoxyamine hydrochloride, N,O-dimethyl hydroxylamine hydrochloride, anhydrous hydrazine and N-methylhydrazine.

Example of general method

2-Amino-6-methoxyamino-9-β-D-ribofuranosylpurine-(JA31)

Synthesis of this compound has been described previously (Ueda, *et al. Chem. Pharm. Bull.*, 1978, **26**, 2122).

The 2-amino-6-chloropurine derivative (302mg; 1mMol), methoxyamine hydrochloride (160mg; 4 equiv.) and triethylamine (0.2 ml) in ethanol (9 ml) were heated overnight at 85°C in a sealed bottle shielded from light. Complete reaction was judged by thin layer chromatography (tlc.) in 20% MeOH-CH_2Cl_2. Evaporation *in vacuo* then trituration with ethanol of the residue gave the product as a white powder (90%) which gave needles on crystallisation from dioxan-water.

In the synthesis of compounds from 6-chloro-9-β-D-ribofuranosylpurine the reaction conditions required lower temperatures and shorter reaction times.

The synthesis of compounds in accordance with general formula I or II has been described in a number of other publications:

JA23, 24, 27 and 30, see Taito *et al*, (1964, Chem. Pharm. Bull. 12, 951);
JA25, see Johnson *et al*, (1958, J. Amer. Chem. Soc. 80, 699);
JA26, see Fuji *et al*, (1991, Chem. Pharm. Bull. 39, 39);
JA28, see Giner-Sorolla *et al*, (1966, J. Med. Chem. 9, 143).
JA32, see Baker *et al*, (1969, J. Med. Chem., 12, 684,687).
JA33, JA34, See Montgomery *et al*, (1957, J. Am. Chem. Soc., 74, 2185).
JA37, see Fujii *et al*, (1983, Chem. Pharm. Bull., 31, 3149-3159).
JA39, see Fujii *et al*, (1971, Chem. Pharm. Bull., 19, 1731).

The analogue 7-deaza-N^6-aminoadenosine (Fig. 20) has been previously prepared and shown to have slight activity against cytomegalovirus (HCMV) (Pudlo *et al*, J. Med. Chem., 1988, 31, 2086-2092). HCMV is a DNA virus, and the mode of action of this compound is suggested to be as an adenosine kinase inhibitor. As such its mode of action is different from that described herein.

The particular compound disclosed by Pudlo *et al* is, in terms of the general formula I used herein, that in which X^1 = CH, X^2 = N, X^3 = NR^6, R^1 = H, R^2 = H and R^6 = H. In preferred embodiments, the present invention thus excludes from its scope a pharmaceutical composition in which the sole active anti-viral compound is that defined immediately above. Since Pudlo *et al* were concerned only with acyclovir analogues (acyclovir being a "selective inhibitor of the virus-encoded DNA polymerase") the prior art does not suggest in any way that the compound disclosed therein might be useful in inhibiting RNA viruses. Moreover the compound in question (1d in Table 1 of Pudlo *et al*) was shown to have an IC_{50} for human foreskin fibroblasts (HFF) of 2μM, whereas the IC_{50} in the plaque reduction assay for cytomegalovirus was 4μM i.e. the compound was more inhibitory for HFF cells than for viral replication, and so of little or no therapeutic usefulness.

Thus in an alternative preferred embodiment the pharmaceutical composition of the present invention preferably comprises an anti-viral active agent which has an IC_{50} in respect of a

conventional RNA virus (especially, for example, polio virus) e.g. as judged by a plaque reduction assay, which is lower than its IC_{50} for HFF cells, according to the assay method disclosed by Pudlo *et al.*

All of the compounds synthesised were recrystallized, characterised by nmr and shown to be substantially pure.

Example 2

Following synthesis, the various compounds were tested *in vitro* for toxicity, by measuring the IC_{50} (i.e. the concentration which caused 50% inhibition) in respect of the inhibitory effects of the compounds on the proliferation of human T-lymphocytes (CEM/O cells). The results are shown below in Table 2.

Table 2

Compound	IC_{50}[a] (μM)
JA21	690 ± 14
JA22	698 ± 11
JA23	622 ± 8
JA24	62 ± 6
JA25	12 ± 3
JA26	44 ± 2
JA27	17 ± 2
JA28	156 ± 15
JA29	16 ± 1
JA30	78 ± 3
JA31	377 ± 62

[a]50% inhibitory concentration.

Example 3

Having established an indication of the toxicity of the various compounds, the ribonucleoside analogues were then tested to determine whether they exhibited any effect on the replication of RNA viruses in *in vitro* cell cultures.

HIV-1 infected CEM cells were subcultured every 4-5 days in the presence of sub-toxic concentrations (in the range of 10-20% of their respective IC_{50} value) of the compounds under test. At each sub-culture, cell-free supernatant (10-20 µl) was transferred to fresh 1ml cell cultures. At regular intervals the cultures were inspected microscopically to assess the extent of any cytopathic effect (giant cell formation). As an alternative, it is also possible to perform an immunoassay to quantify viral p24 production.

The preliminary results for up to 7 passages are shown below in Table 3.

Table 3

Drug	Concentration (µM)	Passage number[a,b]						
		1	2	3	4	5	6	7
JA-21 (dP)	400	100	100	25	50	37	12	6
JA-22 (rP)	400	100	100	100	100	100	100	100
JA-23	400	100	100	12	25	3	0	0
JA-24	10	100	100	25	100	100	100	25
	4	100	100	19	100	100	100	12
JA-25	2	100	100	100	100	100	100	100
	0.8	100	100	87	100	100	100	100
JA-26	10	100	100	25	100	100	12	3
	4	100	100	25	100	100	12	3
JA-27	4	100	100	6	25	25	0	0
JA-28	40	100	100	50	100	100	75	6
	20	100	100	19	100	100	100	100

JA-29	2	100	100	25	100	100	100	100
	0.8	100	100	12	100	100	100	100
JA-30	10	100	100	25	100	100	100	50
JA-31 (rK)	50	100	100	0	0	0	0	0
	20	100	100	3	19	12	0	0
Control (no drug)	-	100	100	25	100	100	100	100

[a]Subcultivation of the drug-treated HIV-1(III_B) exposed CEM cell cultures was performed every 5 days.

[b]Data represent the percentage of cytopathic effect (giant cell formation) as recorded microscopically.

The results show that JA31 (rK) in particular is effective at inhibiting the replication of RNA viruses as exemplified by HIV. Other compounds also appear to be moderately effective: JA23 and JA27 in particular. JA29, mentioned by Loeb *et al*, does not demonstrate any antiviral activity in this assay.

In order to demonstrate that the reduction in viral titer, as evidenced by the decline in observed cytopathic effect, is due to induction of accumulated mutations in the viral genome, proviral DNA will be isolated from the cultures and the sequence of the reverse transcriptase gene determined by routine DNA sequencing reactions. The determined sequence can be compared with the known sequence of the original input virus and the number of mutations calculated relative to those in the virus in the control culture.

Further Studies

Mechanism of action studies will be undertaken to study the effect of the 5'-triphosphate derivatives of the ribonucleotide analogues on human and viral RNA polymerase-catalysed RNA synthesis as well as HIV-1 reverse transcriptase-catalysed conversion of nucleotide analogue-containing RNA to DNA. Also, the substrate affinity of recombinantly produced ribonucleoside kinases for the ribonucleoside analogues and their efficacy of conversion of the ribonucleoside analogues to their 5'-monophosphates will be determined. Insights in the

above-mentioned characteristics of the ribonucleos(t)ide analogues should allow optimisation of the viral mutagenicity of the compounds whilst ideally minimising toxicity, so as to enhance the therapeutic usefulness of the compounds. Masked phosphate derivatives of the ribonucleoside analogues will also be investigated.

Example 4

Other experiments were performed using ribonucleoside analogues present as the phosphorylated ribonucleotide in the presence of transfection agents. For example, the triphosphate of rK, referred to as rKTP, was synthesised as described by Moriyama *et al*, (1998 Nucl. Acids Res. 26, 2105). The triphosphate of rP, rPTP, was prepared in an analogous manner.

These two compounds were then investigated for an inhibitory effect on the replication of HIV in persistently infected Molt4/IIIB cells, or acutely infected MT4/IIIB cells. The compounds were compared with equivalent concentrations of dideoxycytidine (ddC) or dideoxycytosine triphosphate (ddCTP), or a negative control (no drug).

Effect on persistently-infected cells

2nmol of the relevant drug (final concentration 1μM) was mixed with 4μl of liposome DMRIE-C (Gibco BRL) in 800μl of serum-free RPMI 1640 medium (Sigma). After incubating for 45 minutes at room temperature, 10^5 Molt4/IIIB cells in 200μl of serum-free RPMI 1640 medium were added and held at 37°C for 4 hours. At the end of this interval 1ml of RPMI 1640 medium supplemented with 20% serum was added and the mixture cultured at 37°C. at 24hrs, 72hrs and 5 days, aliquots of supernatant were collected and the amount of p24 antigen present was quantified using the Lumipuls™ system (Fuji Rebio). The results are shown in Figure 21.

Effect on acutely-infected cells

10^3 pfu of HIV_{IIIB} were added to 10^5 MT4 cells in 1ml of serum-free RPMI 1640 medium and incubated for 90 minutes at 37°C. The cells were washed three times in serum-free medium and resuspended in 200μl of serum-free medium. Drug administration (100nM

final concentration), culture and p24 assay were then performed as above. The results are shown in Figure 22.

Figure 21 is a graph of viral titer (as measured by amount of p24 antigen in ng/ml) against time (in days), showing the results for cultures of persistently-infected Molt4/IIIB cells with no drug ("Control", triangles), or 1μM final concentration of ddC (open circles), ddCTP (open squares), PTP (filled circles) or rKTP (filled squares). Figure 22 is a graph of p24 antigen (in ng/ml) against time (in days) for cultures of acutely-infected MT4/IIIB cells in the presence of drugs at a final concentration of 100nM, the legend is as for Figure 21.

The results illustrated in Figures 21 and 22 show that both rKTP and rPTP significantly inhibit viral replication compared to controls, and reduce viral titers to levels comparable with known dideoxy chain-terminating compounds which inhibit reverse transcriptase. The ribonucleotide analogues of the invention are believed, however, to be less vulnerable to the evolution of resistant virus strains.

Example 5

Mutations induced on HIV-1 *pol* gene of MT4/IIIB by PTP or KTP

Genomic DNA of MT4/IIIB was collected 3 days after drug administration (final concentration was 100 nM) by DNeasy Tissue Kit (QIAGEN). A part of the *pol* gene (873 bp) was amplified by 2-step polymerase chain reaction (2-step PCR). A first PCR reaction mixture contained 50 pmol of forward primer-1 (5'-GGTACAGTATTAGTAGGACC-3'), 50 pmol of reverse primer-1 (5'-TGTGTCAGTTAGGGTGACAA-3'), 200 μM each dNTP, 5μl of collected genomic DNA, 3 U of *Pfu* DNA polymerase (Promega), 20 mM Tris-HCl pH 8.8 10 mM KCl, 10 mM $(NH_4)_2SO_4$, 2 mM $MgSO_4$, 0.1 % Triton X-100, and 0.1 μg/μl BSA in 50 μl and was divided into five tubes. Each mixture was incubated for 2 min at 95°C. Then it was applied to a thermal cycle reaction comprising 95°C, 1 min; 52°C, 30 sec; and 72°C, 2 min for 45 cycles, followed by incubation for 5 min at 72°C, the cycling controlled by Mastercycler gradient apparatus (Eppendorf).

A second PCR reaction mixture contained 50 pmol of forward primer-2 (5'CAGGGATTAGATATCAGTAC-3'), 50 pmol of reverse primer-2 (5'-TCTCTAACTGGTACCATAAT-3'), 200 μM each dNTP, 1 μl of 1st PCR product from each tube, 1.5 U of *Pfu* DNA polymerase (Promega), 20 mM Tris-HC1 pH 8.8, 10 mM KC1, 10 mM $(NH_4)_2SO_4$, 2 mM $MgSO_4$, 0.1 % Triton X-100, and 0.1 μg/μl BSA in 50 μl and was similarly divided into five tubes. Each mixture was incubated for 2 min at 95°C. Then it was applied to a thermal cycle reaction comprising 95°C, 1 min; 52°C; 30 sec; and 72°C, 2 min. for 30 cycles, followed by incubation for 5 min at 72°C.

Divided 2nd PCR products (total twenty-five tubes for one sample) were collected into one tube, ethanol precipitated, and digested by *Eco*RV and *Kpn*I. After ligation with pBluescriptIISK(+), the constructed plasmid was introduced into *Escherichia coli* DH5α by electroporation. Cloned PCR product was then applied to standard DNA sequencing reaction using forward sequencing primer (5'-AAAGCTGGAGCTCCACCGCG-3') or reverse sequencing primer (5'-AGTGAGCGCGCGTAATACGACTCACTA-TAGGGCGAATTGG-3') and the Thermo Sequenase II dye terminator cycle sequencing kit (Amersham Pharmacia Biotech). Electrophoresis and analysis was carried out by DNA sequencer 378A (Applied Biosystems).

The sequencing revealed that the presence of either rPTP or rKTP increased the mutation frequency, according to the results presented in Table 4 below.

Table 4

	Transition G-to-A	**Transversion T-to-A**	**Total**	**Sequenced (nucleotides)**	**Frequency (x 10^{-3})**
Control	1	2	3	3,113	0.96
PTP	3	6	9	4,809	1.9
KTP	-	6	6	4,642	1.3

Example 6

The inventors constructed an *in vitro* transcription system promoted by HIV 5'-long terminal repeat (LTR) using HeLa nuclear extract supplemented with HIV Tat protein. A 668 bp PCR product from pLTR-*luc* plasmid, which includes HIV 5'-LTR promoter and luciferase gene, was used as a DNA template for a transcription reaction. From this template, 310-mer run-off transcripts were produced. The system is illustrated schematically in Figure 23.

The effect of incorporation of rPTP, at 200μM, in transcription reactions was investigated. The reaction mixture contained conventional nucleotide triphosphates (ATP, GTP, CTP and UTP) at 50μM (the GTP being α^{32}P radio labelled with 10μCi of radioactivity), +/- 200μM PTP, 100ng of template DNA, 40 Units of RNase inhibitor (Wako), 1μl of diluted (1:20) Tat protein and 8 units of HeLa cell nuclear extract in 1x transcription buffer (10mM HEPES pH 7.9, 2mM DTT, 6.25μM $ZnSO_4$, 100mM KCl, 20% glycerol, 4 mM $MgCl_2$). The reaction mixture was incubated for 10 minutes at 30°C and the reaction terminated by adding 7 volumes of stop solution (300mM Tris. HCl pH 7.4, 300mM sodium acetate, 0.5% SDS, 2mM EDTA, 3μg/ml tRNA). Transcripts were then purified by phenol/chloroform extraction and ethanol precipitation. Whole samples were loaded on a 5% polyacrylamide gel and subjected to electrophoresis (40W, for 2 hours). The intensity of the bands corresponding to the 310mer transcripts was measured by a BAS-2000 image analyser (Fujifilm). The intensity of the band in the control reaction (no PTP) was considered to be 100%. The results of the control reaction and the rPTP reaction are shown in Figure 24 below. This shows that the presence of rPTP at 200μM reduced the amount of transcript produced by nearly 50%.

Example 7

The foregoing examples are primarily concerned with demonstrating an inhibitory effect of various ribonucleoside analogues on the replication of HIV. However, as explained above, the compositions of the present invention should also find particular use in combatting infections caused by "conventional" RNA viruses.

In general terms, those skilled in the art can readily ascertain the likely efficacy of various ribonucleoside analogues, by incubating an RNA virus of interest with suitable susceptible host cells in the presence or absence of various concentrations of the ribonucleoside analogue(s) under test, and using an appropriate parameter to measure the amount of viral replication. Suitable parameters might include, for example, an assay of numbers of pfu of virus after a certain length of incubation, or an assay of viral antigen, or amount of cytopathic effect.

A specific example of a suitable screening assay, to identify compounds effective in inhibiting replication of poliovirus, is set forth below. Essentially similar protocols, suitably modified, could be employed to screen for compounds active against other "conventional" RNA viruses.

HeLa cells are propagated in D-MEM/F-12 media (Invitrogen) supplemented with dialyzed fetal bovine serum (2%, Invitrogen). For poliovirus infection assays, cells are plated in 24-well dishes (1 X 10^5 cells/well) 48 h before the experiment, test compounds are preloaded 24 hours before the experiment, and cells are infected with 2000 pfu poliovirus per well. Upon reaching 100% cytopathic effect (CPE), virus is harvested by freeze-thaw and serial dilutions are plaqued on 6-well dishes of confluent HeLa S3 cells. After 72 hours, cells are stained with Crystal Violet (0.2% in 20% ethanol) to visualize plaques. Time to 100% CPE is recorded as the number of days required for poliovirus (2000 pfu) to cause visibly complete cell death.

Example 8

General synthesis of triphosphates

To a solution of 50mg of either 6-chloropurine riboside triphosphate or 2-amino-6-chloropurine riboside triphosphate in 0.5 cm^3 of water was added the hydroxylamine or hydrazine derivative (5 equivalents) and the reaction stirred at room temperature for 4 hours. The synthesis of the 2-amino derivatives required reaction at 40°C for 4 hours. The solution was lyophilised, dissolved in water, and purified by HPLC. HPLC (Phenomenex Luna (10 μm diameter particle size) C-18 reverse phase column, buffer A, 0.1 M TEAB;

buffer B, 0.1 M TEAB, 50% MeCN) % to 40% buffer B over 40 minutes at 8 ml/min.). Samples were evaporated and converted to their sodium salts by passage through a Dowex 50WX4-200 resin (Na^+ form).

JA23 5'-triphosphate Yield 49mg. δ_P (D_2O) -4.40 (d, γ-P), -9.73 (d, α-P), -20.22 (t, β-P).
JA24 5'-triphosphate Yield 20mg. δ_P (D_2O) -4.56 (d, γ-P), -9.80 (d, α-P), -20.43 (t, β-P).
JA26 5'-triphosphate Yield 30mg. δ_P (D_2O) -4.71 (d, γ-P), -9.79 (d, α-P), -20.36 (t, β-P).
JA27 5'-triphosphate Yield 27mg. δ_P (D_2O) -4.41 (d, γ-P), -9.74 (d, α-P), -20.22 (t, β-P).
JA28 5'-triphosphate Yield 24mg. δ_P (D_2O) -4.49 (d, γ-P), -9.77 (d, α-P), -20.35 (t, β-P).
JA31 triphosphate Yield 24mg. δ_P (D_2O) -4.51 (d, γ-P), -9.77 (d, α-P), -20.36 (t, β-P).

Example 8A

Incorporation of nucleoside analogues into sym/sub-U and sym/sub-C by Polio virus strain 3D (PV3D)

This was carried out generally as previously described (Crotty *et al*, Proc. Natl. Acad. Sci. USA, 2001, 98, 6895-6900; Crotty *et al*, Nature Medicine, 2000, 6, 1375-1379).

All nucleotide and nucleoside incorporation experiments were carried out in a reaction buffer containing 50 mM HEPES pH=7.5, 5 mM $MgCl_2$, 10 mM beta-mercaptoethanol, and 60 μM $ZnCl_2$ and using short synthetic primer/template systems comprising either uracil or cytidine as the first base opposite which incorporation is to take place (referred to as sym/sub-U or sym/sub-C respectively). The assay was performed by incubating PV 3D polymerase (2 μM) with either sym/sub-U or sym/sub-C (1 μM) for 90 seconds at 30°C and the reaction was initiated by the addition of the nucleotide or analogue (500 μM). Reactions were stopped by quenching with EDTA (50 mM final concentration) 2 minutes after initiation.

Analysis of the products formed in the reaction was done using a 23% PAGE denaturing gel. Gels were visualized by using a PhosphorImager and quantitated using the ImageQuant software.

Sequence of sym/sub-U primer/template:

5'-GCAUGGGCCC

CCCGGGUACG-5'

Sequence of sym/sub-C primer/template:

5'-GAUCGGGCCC

CCCGGGCUAG-5'

The results are shown in Figures 25 A/B and 26 AB. Figures 25A and 26A are images of PAGE analysis of the products obtained using the sym/sub-U and sym/sub-C systems respectively. Figures 25B and 26B are pictorial representations of the results in bar chart form. The height of the bars indicates the amount of product obtained (in arbitary units) relative to control reaction mixtures comprising ATP nucleotide. The JA numbers refer to the respective 5'-triphosphates.

Figures 25A/B show that incorporation of all compounds into sym/sub-U by PV $3D^{pol}$ takes place to a similar extent as the correct nucleotide, ATP. Figure 25A shows a 23% PAGE denaturing gel with the products formed in the reaction. The template is the major band at the bottom of the gel. The extended product(s) is represented by the fainter band(s) higher up the gel. Figure 25B shows the amount of product being formed when the inhibitors were used in comparison with the incorporation of AMP.

JA numbers refer to their 5'-triphosphates.

The results with sym/sub-C are shown in Figures 26 A/B. In the case of sym/sub-C, incorporation of the analogues occurs in a similar scale as the incorporation of GMP with the exception of JA-26, for which the efficiency of incorporation is nearly half that of GMP. The reaction with sym/sub-C shows the formation of a product 12-nucleotides long as the major product. This is the result of incorporation of the analogues opposite both cytidine and uracil located next to each other. In the case of sym/sub-U, above, uracil is followed by adenosine, which appears not to be a good 'template' for the analogues. JA-

28 shows products of incorporation 12- and 13-nucleotides long. This indicates that JA-28 may be both a purine and a pyrimidine analogue.

Example 9

General synthesis of nucleobases.

To a solution of either 2-chloropurine or 2-chloro-6-aminopurine in water was added 10 equivalents of reagent (e.g. methoxyamine, methyl hydrazine, in the case of hydrochloride salts, e.g. hydroxylamine hydrochloride, 10 equivalents of triethylamine are also added) and the solution heated at 60°C for 1-48 hours (2-chloropurine reacts faster than 2-chloro-6-aminopurine). On cooling the product precipitates and is isolated by filtration.

Example 10

General synthesis of masked phosphate derivatives.

10.1 The general structure of cyclosal derivatives is shown in Figure 27, wherein X is H or CH_3 and Y is Cl or H.

Using a mixture of DMF and acetonitrile as solvent, DIPEA as base and the 3-methylchlorophosphane or 5-chlorochlorophosphane, the nucleosides were prepared according to Meier *et al* (Eur. J. Org. Chem., 1998, 837-846) and then oxidised with t-BuOOH.

10.2 The general structure of SATE derivatives is shown in Figure 28.

Bis(*S*-pivaloyl-2-thioethyl) *N,N*-diisopropylphosphoramidite was prepared as described by Lefebvre *et al*, (J. Med. Chem., 1995, 38, 3941-3950). Turbo-Tet (ethylthiotetrazole) (3 equivalents) was added to a stirred solution of the ribonucleoside analogue and bis(*S*-pivaloyl-2-thioethyl) *N,N*-diisopropylphosphoramidite (1.2 equivalents) in a mixture of DMF and THF. The solution was stirred at room temperature for 30 mins. The solution was then either cooled to -40°C and a solution of 3-chloroperbenzoic acid (1.3 equivalents) in dichloromethane added, and the solution allowed to warm to room temperature over 1 hour. Sodium sulfite was then added to neutralise the 3-chloroperbenzoic acid.

Alternatively the reaction mixture was treated with iodine oxidation solution (DNA synthesis grade). The reaction mixture was concentrated, dissolved in ethyl acetate, washed with aqueous sodium bicarbonate and evaporated. The product was purified by column chromatography.

APPENDIX

Table 1

The Negative Stranded ssRNA Viruses

Order / Family / *Subfamily*	*Genus*	*Type Species*	*Host*
Mononegavirales			
Bornaviridae			
	Bornavirus	*Borna disease virus*	Vertebrates
Filoviridae	"Ebola-like viruses"	*Ebola virus*	Vertebrates
	"Marburg-like viruses"	*Marburg virus*	Vertebrates
Paramyxoviridae			
Paramyxovirinae			
	Respirovirus	*Human parainfluenza virus 1*	Vertebrates
	Morbillivirus	*Measles virus & rinde pest virus*	Vertebrates
	Rubulavirus	*Mumps virus*	Vertebrates
Pneumovirinae			
	Pneumovirus	*Human respiratory syncytial virus*	Vertebrates
	Metapneumovirus	*Turkey rhinotracheitis virus*	Vertebrates
Rhabdoviridae			
	Vesiculovirus	*Vesicular stomatitis Indiana virus*	Vertebrates
	Lyssavirus	*Rabies virus*	Vertebrates
	Ephemerovirus	*Bovine ephemeral fever virus*	Vertebrates
	Novirhabdovirus	*Infectious hematopoietic necrosis virus*	Vertebrates
	Cytorhabdovirus	*Lettuce necrotic yellow virus*	Plants
	Nucleorhabdovirus	*Potato yellow dwarf virus*	Plants

Orthomyxoviridae			
	Influenzavirus A	*Influenza A virus*	Vertebrates
	Influenzavirus B	*Influenza B virus*	Vertebrates
	Influenzavirus C	*Influenza C virus*	Vertebrates
	Thogotovirus	*Thogoto virus*	Vertebrates
Bunyaviridae			
	Bunyavirus	*Bunyamwera virus*	Vertebrates
	Hantavirus	*Hantaan virus*	Vertebrates
	Nairovirus	*Nairobi sheep disease virus*	Vertebrates
	Phlebovirus	*Sandfly fever Sicilian virus*	Vertebrates
	Tospovirus	*Tomato spotted wilt virus*	Plants
	Tenuivirus	*Rice stripe virus*	Plants
	Ophiovirus	*Citrus psorosis virus*	Plants
Arenaviridae			
	Arenavirus	*Lymphocytic choriomeningitis virus*	Vertebrates
	Deltavirus	*Hepatitis delta virus*	Vertebrates

The Positive Stranded ssRNA Viruses

Order / Family / *Subfamily*	*Genus*	*Type Species*	*Host*
Narnaviridae			
	Narnavirus	*Saccharomyces cerevisiae 20S narnavirus*	Yeast
	Mitovirus	*Cryphonectria parasitica NB631 virus*	Yeast
Leviviridae	*Levivirus*	*Enterobacteria phage MS2*	Bacteria

	Allolevivirus	*Enterobacteria phage Qβ*	Bacteria
Picornaviridae			
	Enterovirus	*Poliovirus 1*	Vertebrates
	Rhinovirus	*Human rhinovirus 1A*	Vertebrates
	Hepatovirus	*Hepatitis A virus*	Vertebrates
	Cardiovirus	*Encephalomyocarditis virus*	Vertebrates
	Aphthovirus 1AH	*Food-and-mouth disease virus O*	Vertebrates
	Parechovirus	*Human echovirus 22*	Vertebrates
	"Cricket paralysis-like viruses"		
		Cricket paralysis virus	Invertebrates
Sequiviridae			
	Sequivirus	*Parsnip yellow fleck virus*	Plants
	Waikavirus	*Rice tungro spherical virus*	Plants
Comoviridae			
	Comovirus	*Cowpea mosaic virus*	Plants
	Fabavirus	*Broad bean wilt virus 1*	Plants
	Nepovirus	*Tobacco ringspot virus*	Plants
Potyviridae			
	Potyvirus	*Potato virus Y*	Plants
	Rymovirus	*Ryegrass mosaic virus*	Plants
	Macluravirus	*Maclura mosaic virus*	Plants
	Ipomovirus	*Sweet potato mild mottle virus*	Plants
	Bymovirus	*Barley yellow mosaic virus*	Plants
	Tritimovirus	*Wheat streak mosaic virus*	Plants
Caliciviridae			
	Vesiculovirus	*Swine vesicular exanthema virus*	Vertebrates
	Lagovirus	Rabbit hemorrhagic disease virus	Vertebrates
	"Norwalk-like viruses"	*Norwalk virus*	Vertebrates
	"Sapporo-like viruses"	*Sapporo virus*	Vertebrates

	"Hepatitis E-like viruses"	*Hepatitis E virus*	Vertebrates
Astroviridae	*Astrovirus*	*Human astrovirus 1*	Vertebrates
Nodaviridae			
	Alphanodavirus	*Nodamura virus*	Invertebrates
	Betanodavirus	*Striped jack nervous necrosis virus*	Vertebrates
Tetraviridae			
	Betatetravirus	*Nudaurelia capensis β virus*	Invertebrates
	Omegatetravirus	*Nudaurelia capensis ω virus*	Invertebrates
	Sobemovirus	*Southern bean mosaic virus*	Plants
Luteoviridae			
	Luteovirus	*Barley yellow dwarf virus*	Plants
	Polerovirus	*Potato leafroll virus*	Plants
	Enamovirus	*Pea enation mosaic virus 1*	Plants
	Umbravirus	*Carrot mottle virus*	Plants
Tombusviridae			
	Tombusvirus	*Tomato bushy stunt virus*	Plants
	Carmovirus	*Carnation mottle virus*	Plants
	Avenavirus	*Oat chlorotic stunt virus*	Plants
	Aureusvirus	*Pothos latent virus*	Plants
	Necrovirus	*Tobacco necrosis virus*	Plants
	Dianthovirus	*Carnation ringspot virus*	Plants
	Machlomovirus	*Maize chlorotic mottle virus*	Plants
	Panicovirus	*Panicum mosaic virus*	Plants
Nidovirales			
Coronaviridae			
	Coronavirus	*Avian infectious bronchitis virus*	Vertebrates
	Torovirus	*Berne virus*	Vertebrates
Arteriviridae			

	Arterivirus	*Equine arteritis virus*	Vertebrates
Flaviviridae			
	Flavirus	*Yellow fever virus*	Vertebrates
	Pestivirus	*Bovine diarrhea virus*	Vertebrates
	Hepacivirus	*Hepatitis C virus*	Vertebrates
Togaviridae			
	Alphavirus	*Sindbis virus*	Vertebrates
	Rubivirus	*Rubella virus*	Vertebrates
	Tobamovirus	*Tobacco mosaic virus*	Plants
	Tobravirus	*Tobacco rattle virus*	Plants
	Hordeivirus	*Barley stripe mosaic virus*	Plants
	Furovirus	*Soil-borne wheat mosaic virus*	Plants
	Pomovirus	*Potato mop-top virus*	Plants
	Pecluvirus	*Peanut clump virus*	Plants
	Benyvirus	*Beet necrotic yellow vein virus*	Plants
Bromoviridae			
	Alfamovirus	*Alfalfa mosaic virus*	Plants
	Ilarvirus	*Tobacco streak virus*	Plants
	Bromovirus	*Brome mosaic virus*	Plants
	Cucumovirus	*Cucumber mosaic virus*	Plants
	Oleavirus	*Olive latent virus 2*	Plants
	Ourmiavirus	*Ourmia melon virus*	Plants
	Idaeovirus	*Raspberry bushy dwarf virus*	Plants
Closteroviridae			

	Closterovirus	*Beet yellows virus*	Plants
	Crinivirus	*Lettuce infectious yellows virus*	Plants
	Capillovirus	*Apple stem grooving virus*	Plants
	Trichovirus	*Apple chlorotic leaf spot virus*	Plants
	Vitivirus	*Grapevine virus A*	Plants
	Tymovirus	*Turnip yellow mosaic virus*	Plants
	Carlavirus	*Carnation latent virus*	Plants
	Potexvirus	*Potato virus X*	Plants
	Allexivirus	*Shallot virus X*	Plants
	Foveavirus	*Apple stem pitting virus*	Plants
Barnaviridae			
	Barnavirus	*Mushroom bacilliform virus*	Fungi
	Marafivirus	*Maize rayado fino virus*	Plants

The dsRNA Viruses

Order Family *Subfamily*	*Genus*	*Type Species*	*Host*
Cystoviridae	*Cystovirus*	*Pseudomonas phage Φ6*	Bacteria
Reoviridae			
	Orthoreovirus	*Reovirus 3*	Vertebrates

	Orbirirus	*Bluetongue virus 1*	Vertebrates
	Rotavirus	*Simian rotavirus SA11*	Vertebrates
	Coltivirus	*Colorado tick fever virus*	Vertebrates
	Aquareovirus	*Golden shiner virus*	Vertebrates
	Cypovirus	*Bombyx mori cypovirus 1*	Invertebrates
	Fijivirus	*Fiji disease virus*	Plants
	Phytoreovirus	*Wound tumor virus*	Plants
	Oryzavirus	*Rice rattged stunt virus*	Plants
Birnaviridae			
	Aquabirnavirus	*Infectious pancreatic necrosis virus*	Vertebrates
	Avibirnavirus	*Infectious bursal disease virus*	Vertebrates
	Entomobirnavirus	*Drosphila X virus*	Invertebrates
Totiviridae			
	Totivirus	*Saccharomyces cerevisiae virus L-A*	Fungi
	Giardiavirus	*Giardia lamblia virus*	Protozoa
	Leishmaniavirus	*Leishmania RNA virus 1-1*	Protozoa
Partitviridae			
	Partitvirus	*Gaeumannomyces graminis virus 019/6-A*	Fungi
	Chrysovirus	*Penicillium chrysogenum virus*	Fungi
	Alphacryptovirus	*White clover cryptic virus 1*	Plants
	Betacryptovirus	*White clover cryptic virus 2*	Plants
Hypoviridae			
	Hypovirus	*Cryphonectria hypovirus 1-EP713*	Fungi
	Varicosavirus	*Lettuce big-vein virus*	Plants